INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

Phytochemistry, Antibacterial activity and Chromosome number of two species of *Daphne* from *Algeria*

Messaoud Ramdani[1*], Takia Lograda[1], Pierre Chalard[2,3] and Gilles Figueredo[4]

[1]Laboratory of Natural Resource Valorisation, Faculty of Natural Sciences and Life, Ferhat Abbas University, 19000 Setif, Algeria

[2]Clermont Université, ENSCCF, Institut de Chimie de Clermont-Ferrand, BP 10448, F-63000 Clermont-Ferrand, France.

[3]CNRS, UMR 6296, ICCF, F-63171 Aubière, France.

[4]LEXVA Analytique, 460 rue du Montant, 63110 Beaumont, France.

ABSTRACT

The chemical composition of essential oil isolated from *Daphne gnidium* and *D. laureola* by hydrodistillation, was analysed by GC and GC/MS. A total 31 compounds representing 85.2% of the oil were identified in *D. gnidium*, and 47 components representing 91.3% of the total oil in *D. laureola*. The chemical composition of *D. gnidium* and *D. laureola*, is very different, the only common component is palmetic acid. *D. gnidium* is characterized by palmetic acid, Eicosene, linoleic acid and Dodecane. While *D. laureola* contains the major products; palmitic acid, thymol, Dremenin, phytol acetat, tritriacontane and α-pinene. The essential oil of *D. gnidium* has low activity against *Escherichia coli* and no effect on *Bacillus cereus, Micrococcus luteus, Staphylococcus aureus* and the yeast *Saccharomyces cerevisiae*. The essential oil of *D. laureola* of the two populations showed low activity against *Micrococcus luteus* and *Escherichia coli,* while *Staphylococcus aureus, Bacillus cereus* and *Saccharomyces cerevisiae* show significant resistance to this oil. The population of *D. gnidium* and *D. laureola* studied showed a diploid chromosome number 2n = 2x = 18.

KEY WORDS: *Daphne gnidium, D. laureola,* Essential oil, antibacterial activity, Chromosome, Algeria.

INTRODUCTION

Several studies of *Daphne* species showed the presence of coumarin[1-4], flavonoids[5-7], lignans[1, 2, 8, 9] and daphnane diterpene esters[10-13]. The study of chemical extracts from the leaves of *Daphne gnidium* has shown the presence of flavonoids, tannins, coumarins and polyphenols[14-19].

The chemical composition of the essential oil of *D. genkwa* of China shows the presence of the α-santalene, methyl eugenol, elemicin, δ-cadinene, β-caryophyllene, α-copaene, β-santalene and nerolidol[20]. In Turkey the essential oil of *D. oleoides* contains nonacosane, hexadecanoic acid, tetradecanoic acid, heptacosane, phytol and pentacosane[21]. The chemical composition of *D. pontica* showed the presence of major compounds: acetone hexahydrofarnesyl, carvacrol, dihydroedulane II, Geranyl acetone, thymol and nonacosane[21].

The *Daphne* species exhibit a wide range of biological activity, used as anti-leukemic[22], Neurotrophic[23, 24], Anti-hyperglycemic[25], anticancer[22, 26]. They are used in the treatment of rheumatoid arthritis and of apoplexy[12, 27], and in the treatment of skin disorders[28, 29], in the healing of wounds[30], in malaria and inflammation[31, 32]. They exhibit antiviral activity[33], and have sterilizing effects[34], antibacteria[35] and are also considered pesticides[36]. The flower buds are used as a diuretic, expectorant[37] and anti-cholesterol[24].

The study of chemical extracts from the leaves of *Daphne gnidium* has shown the presence of products responsible for the antioxidant activity[15-17]. *D. gnidium* is used against hepatitis[38]. The extracts from the leaves and bark have antibacterial and antifungal activity[39-41]. The powder of the roots was used as an abortifacient and bark as a diuretic agent and for treating teeth[18].

The bark and fruits of *D. laureola* are toxic to humans[42]. Based on a survey conducted in the region of Beni aziz and Oued Elberd the local populations, use *D. laureola* as plaster for fractures bones and against sterility.

The study of the antibacterial activity of essential oil of *D. oleifolia* performed by Tayoub *et al*[43], on several bacterial strains, has shown that *Bacillus* sp is very sensitive while *Klebsiella* sp is more resistant. The essential oil of *D. cneorum* shows strong activity against several microorganisms and low activity against *Proteus mirabilis*[44]. The oil extracted from the roots of *D. mucronata* is effective against *E. coli* and *B. subtilis* while extracts of stems and leaves are inactive against *Pseudomonas aeruginosa*[45].

Cytologically the family of *Thymelaeaceae* is homogeneous, the genres *Daphne*, *Edgeworthia* and *Wikstoemia*, have a basic chromosome number of x = 9 [46, 47]; *Daphne pontica* and *D. Mezereum* have a diploid chromosome number 2n = 18 [48]. The chromosome number 2n = 18 was reported for the species *D. gnidium*[47, 49, 50]. The chromosome number of *D. laureola* in Bulgaria is 2n = 18 [48, 51-53]. In Spain Löve and Kjellqvist[54] reported a chromosome number of 2n = 18 for *D. laureola*.

MATERIALS AND METHODS

Plant material

Two species of *Daphne* were collected from natural populations of Setif region, located in the North East of Algeria. Aerial parts were collected in September 2013, of three localities (BeniAziz and Oued Elbared for *D. laureola* and Amouchas for *D gnidium*) (Figure 1). Voucher specimens were deposited in the herbarium of the Department of Ecology and Biology, Ferhat Abbas University Sétif 1, Algeria.

The genus *Daphne* L. is represented by three species in Algeria, *D. oleoides* Schreb, *D. gnidium* L. and *D. laureola* L., they are found in the scrub of the Algerian Tell[19, 55].

Daphne gnidium commonly named Lazzaz (in Arabic); it is a tree or shrub that grows in the Mediterranean region. The leaves very dense are lanceeoleat-linear, 5-7 mm wide. The inflorescences are terminal, entirely white-tomentose. The fruit is a berry[55] (Figure 2a).

Daphne laureola (Ajiji in Arabic), is a sub-shrub with large leaves (6-12 cm), alternate, spirally arranged, dark and bright green on top and lighter on the underside. The flowers are greenish-yellow (Figure 2b).The fruits are drupes or bluish-black berry, between 8-13 mm in length. *D. laureola* is located in Algeria on high mountains[56].

Extraction of the essential oil

The air-dried aerial parts of the three populations were subjected to hydro-distillation for 3 h with the distilled water using a Clevenger-type apparatus. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in screw capped glass vials in a refrigerator at 4-5°C, prior to analysis. Yield based on dried weight of the samples was calculated.

Essential oil analysis

The essential oils were analysed on a Hewlett-Packard gas chromatograph Model 5890, coupled to a Hewlett-Packard model 5971, equipped with a DB5 MS column (30 m X 0.25 mm; 0.25 µm), programming from 50°C (5 min) to 300°C at 5°C/min, with a 5 min hold. Helium was used as the carrier gas (1.0 ml/min); injection in split mode (1:30); injector and detector temperatures, 250 and 280°C, respectively. The mass spectrometer worked in EI mode at 70 eV; electron multiplier, 2500 V; ion source temperature, 180°C; MS data were acquired in the scan mode in the m/z range 33-450. The identification of the components was based on comparison of their mass spectra with those of NIST mass spectral library[57, 58] and those described by Adams, as well as on comparison of their retention indices either with those of authentic compounds or with literature values[59].

Antibacterial and antifungal Activities

The antimicrobial activity of *Daphne gnidium* and *D. laureola* essential oils has been investigated on different bacteria and yeast. The Extract Essential oil was tested against the following bacteria; two gram negative bacteria: *Escherichia coli* ATCC 25922 and *Bacillus cereus* ATCC 10876 and two gram positive bacteria; *Staphylococcus aureus* ATCC 6538 and

Micrococcus luteus ATCC 533 and the yeast *Saccharomyces cerevisiae* ATCC 763. The *in vitro* antibacterial and antifungal activity of the examined extract was assessed the determination of the activity by the disk diffusion method, according to recommendations of the Clinical and Laboratory Standards Institute. The bacterial inocula were prepared from overnight broth culture in physiological saline (0.9 % of NaCl) in order to obtain an optical density ranging from 0.08-01 at 625 nm. Muller-Hinton agar (MH agar), and the Sabouraud broth for yeast, were poured in Petri dishes, solidified and surface dried before inoculation. Sterile discs (6 mm Φ) were placed on inoculated agars, by test bacteria, filled with 10 µl of mother solution. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. All tests were performed in triplicate. Then, Petri dishes were incubated at 37°C during 18 to 24h aerobically (Bacteria). After incubation, inhibition zone diameters were measured and documented.

Karyology

For karyotypic analysis, the squashing method is used. The root-tip meristems from germinating seeds were usually used for chromosome preparations. A pre-treatment at room temperature for 1.5 h was usually applied before fixation of the root-tips, in a 0.05% water solution of colchicine. After fixation in a cold mixture of ethanol acetic acid (3:1), the root-tips were stored in 70° ethanol and at a low temperature, until used. The following procedure involved the maceration in 45% acetic acid for 15 min. The following procedure involved the maceration in 45% acetic acid for 15 min. staining of chromosomes was made of emerging root-tips in acetic orcein with heating for one minute. Cutting off the meristems and squashing them in a drop of orcein.

RESULTS

Essential oils of *Daphne laureola* and *D. gnidium* have a transparent color. The average yield of essential oil is 0.05%. The extraction of essential oils was performed until a sufficient amount is obtained, then analyzed by GC-GC / MS (Figure 3). The chemical components of the essential oils identified for both species are presented according to their appearance in the chromatograms (Table 1).

32 compounds were identified in the essential oil of *D. gnidium*, which corresponds to 87.66% of the total oil. The hexadecanoic acid is the major constituent in the oil of this species with a rate of 20.97%, followed by eicosene (12.49%), decanal (5.03%) and tridecanal (4 55%). In the essential oil of *D. Laureola*, 40 and 35 components were identified, corresponding to a percentage of 87.86% and 96.81% of the two populations of Beni Aziz and Oued Elberd respectively. In the Beni Aziz population the major compounds in *D. laureola* are thymol (31.75%), hexadecanoic acid (6.19%), dodecanal aldyhyde (4.08%) and neryl acetone (3.56%), while Oued Elberd population is characterized by thymol (16.73%), drimenin (15.76%) and the hexadecanoic acid (9.47%). The chemical composition of Beni Aziz population differs from Oued Elberd population by the presence of 24 terpene components. 13 compounds are present in the essential oil of both species (*D. gnidium* and *D. laureola*). *D. gnidium* is characterized by the presence of five components (tetradicane, γ-terpinene, tetracosane, octacosane, and methyl salicylate) are absent in the *D. laureola* oil.

The antibacterial activity of essential oils of *D.gnidium* and *D.laureola* is evaluated by the disc method. The results are expressed by measuring the halos of inhibition diameter, after 24 hours of incubation at 37°C (table 2). The results show that the essential oil of *D. gnidium* has low activity against *Escherichia coli* and no effect on *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus*, as well as the yeast *Saccharomyces cerevisiae*. The essential oil of *D. laureola* of the two stations showed low activity against *Micrococcus luteus* and *Escherichia coli*, while *Staphylococcus aureus*, *Bacillus cereus* and *Saccharomyces cerevisiae* have shown a high resistance to *D. laureola* essential oils (Figure 4). The observations of root cells meristematic at metaphase of *Daphne gnidium* and *D. laureola* gave a diploid chromosome number 2n = 2x = 18 (Figure 5).

DISCUSSION

An average yield of 0.05 obtained for *Daphne* species is low compared to other species (0.1-0.35) for *Rosmarinus*[60]. The chemical composition of essential oils of species (*D. laureola* and *D. gnidium*) is very diverse and heterogeneous. The chemical profile differs from those reported in other species of the genus.

The major components in the essential oil of *D. oleoides* of Turkey are identified, the nonacosane, hexadecanoic acid, tetradecanoic acid, phytol and heptacosane[21]. The same authors on the essential oils of *D. pontica* have identified the major components (hexahydrofarnesyl acetone, carvacrol, dihydroedulane II (E), Geranyl acetone, thymol and nonacosane). The furfural, α-copaene, α-santalene, β-caryophyllene, β-santalene, δ-cadinene, methyl eugenol, nerolidol and elemicin are identified as major products of *D. genkwa* essential oil from China[20].

The essential oils of the species studied have shown a very low antibacterial activity. Studying of Tayoub has shown that strain *Bacillus* sp is very sensitive to the essential oil of *D. oleifolia*[43], while *Klebsiella* sp is very resistant. The methanol extract of *D. cneorum* exhibits strong activity against *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and low activity against *Proteus mirabilis*[44]. The ethanolic extract of the roots of *Daphne mucronata* showed an antibacterial activity against *Escherichia coli* and *Bacillus subtilis*, while the extract of stems and leaves are inactive against *Pseudomonas aeruginosa*[45].

On samples of Megres population we counted a chromosome number of 2n = 18 for *D. gnidium*, the same results are reported in Spain[47, 50]. *D. laureola* shows a diploid with 2n = 18, these results are consistent with those found in Bulgaria[48] and Spain[54]. This chromosome number 2n = 18 is reported for the first time for the Algerian samples and confirms those published by Goldblatt[51-53]. The *Thymelaeaceae* family is a homogeneous group with a basic chromosome number x = 9 [46], this allows us to say that the basic chromosome number of the genus *Daphne* at least in Algeria is x = 9.

CONCLUSION

Analysis of the chemical composition of essential oils by GC-GC / MS allowed the identification of 32 components in the essential oil of *Daphne gnidium*, with hexadecanoic acid the major compound. 40 and 35 terpene components are identified in the essential oils of two populations of *Daphne lauroela*, Beni Aziz and Oued Elberd. The major component in these oils is thymol.

The antibacterial activity of the species studied showed that the essential oil of *Daphne laureola* has a very low activity on *Escherichia coli* and *Bacillus cereus* and no effect on *Micrococcus luteus* and *Staphylococcus aureus*, as well as the yeast *Saccharomyces cerevisiae*. The essential oil of *D. gnidium* shows low activity against *Escherichia coli*, and no activity against *Staphylococcus aureus*, *Bacillus cereus*, *Micrococcus luteus* and *Saccharomyces cerevisiae*.

The chromosome counts were focused on meristematic cells of *D. gnidium* and *D. laureola*. Our results have allowed us to determine the diploid chromosome number 2n = 2x = 18 in both species with a basic chromosome number x = 9.

ACKNOWLEDGEMENTS

The work was supported by VRBN laboratory, Ferhat Abbas University (Sétif 1) and Chemical Laboratory of carbohydrates Heterocyclic of Clermont Ferrant, France.

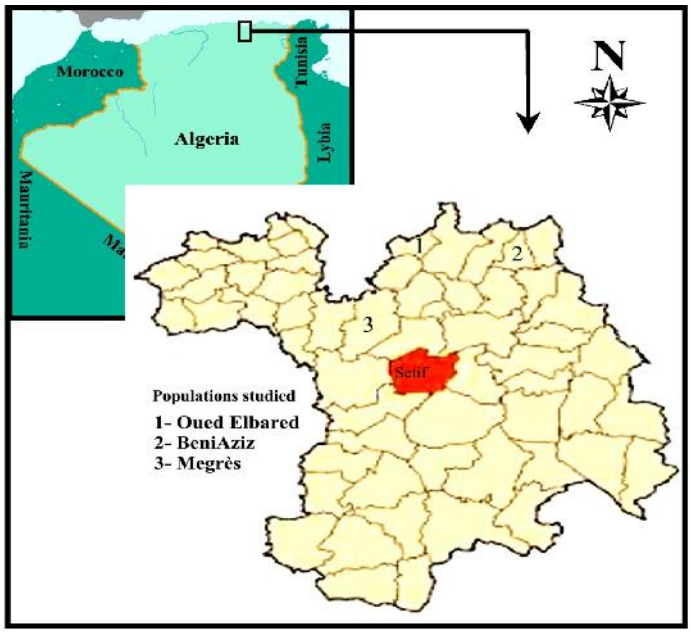

Figure 1
Stations sampled; (1 and 2) *D. laureola*, (3) *D. gnidium*

Figure 2
***Daphne gnidium* from Megrès and *D. laureola* from BeniAziz)**

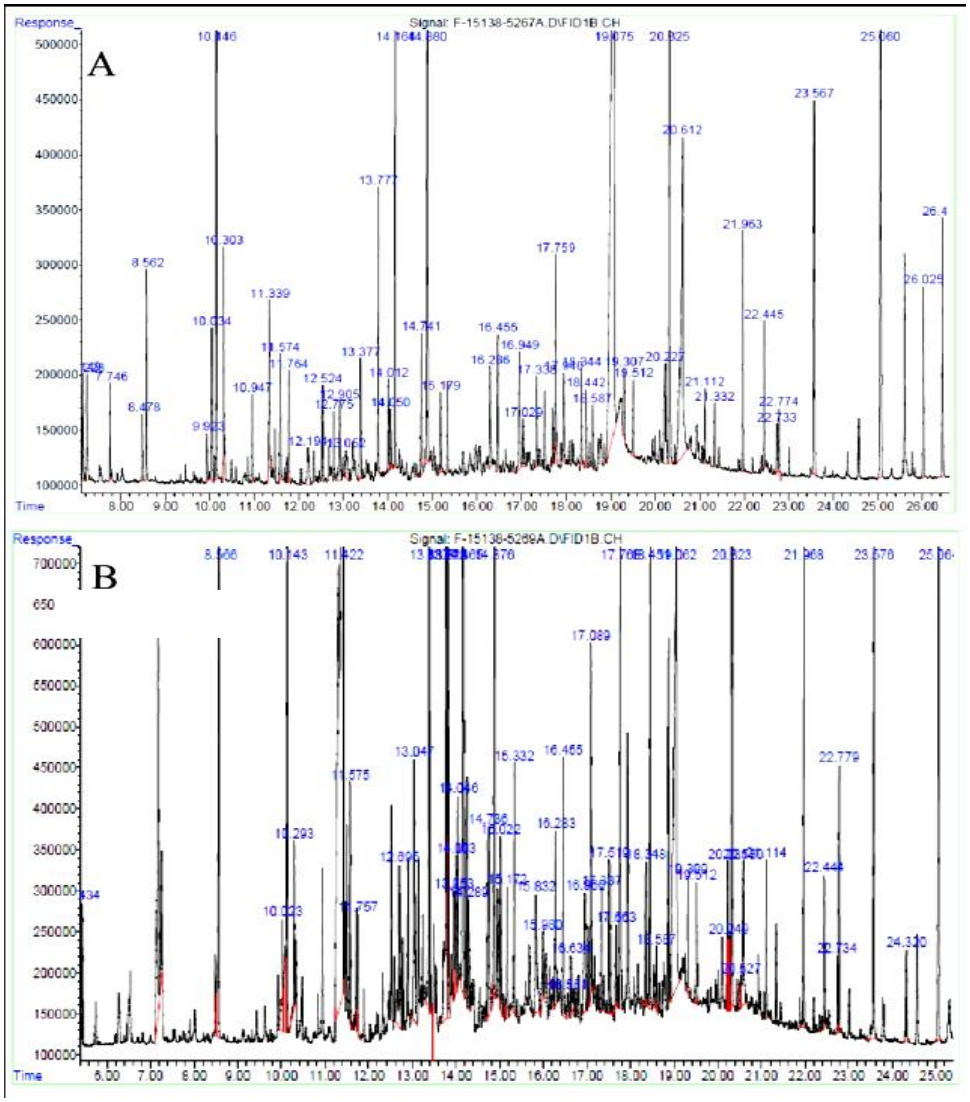

Figure 3
Profile GC / FID of essential oils of *Daphne*
(A: *D. gnidium; B: D. laureola*)

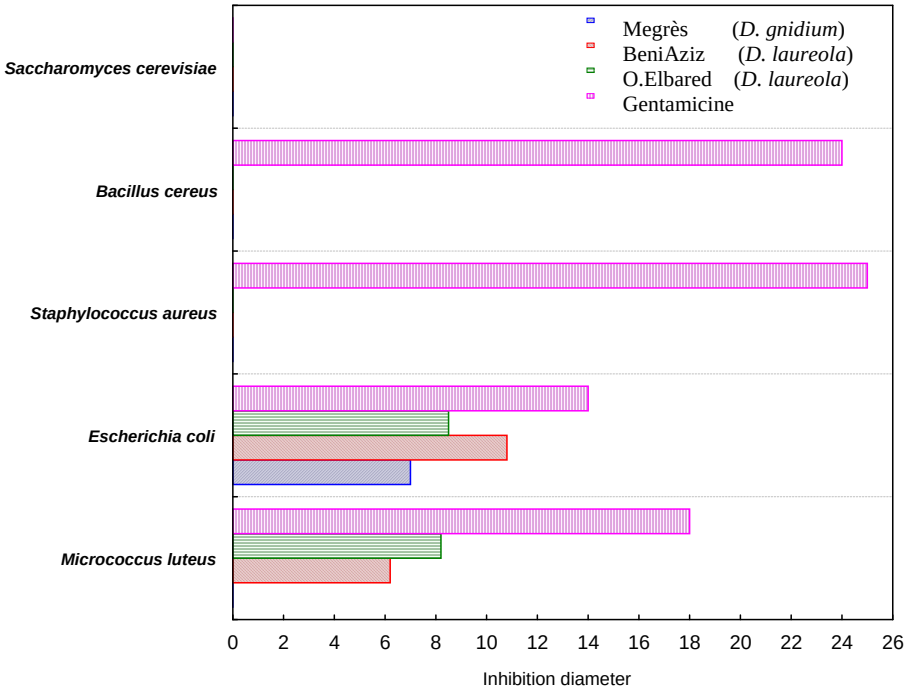

Figure 4
Antibacterial and antifungal activity of *Daphne* species essential oils

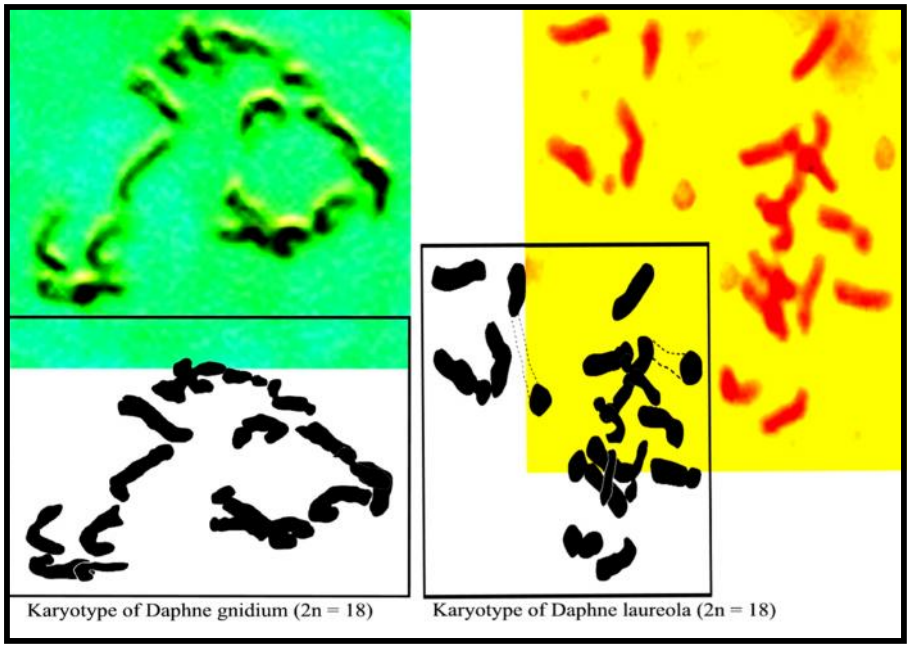

Figure 5
Karyotypes of *Daphne gnidium* and *D. laureola* (2n = 2x = 18)

Table 1
Chemical composition of two species of *Daphne*

Species		*gnidium*	*laureola*	
Populations		Megrès	Beni aziz	O. El-Bared
Yield (v/v)		0.05	0.04	0.06
Number of compounds		31	47	33
Total (%)	KI	85.2	86.6	96.1
2-heptane	863	0	0.59	0
α-pinene	934	0	0	4.31
β-pinene	978	0	0	0.89
Cymene-ortho	1025	0.81	2.96	1.03
Limonene	1030	0.99	0	1.31
γ-terpinene	1059	0.66	0	0
Linalool	1099	0.51	0	1.42
Nonanal	1105	1.46	2.13	2.02
α-campholenal	1128	0	0	0.66
Methyl salicylate	1193	0.38	0	0
Dodecane	1204	5.03	3.57	1.13
Neryl formate	1220	1.31	0.56	1.33
Thymol	1291	1.40	31.75	16.73
Carvacrol	1301	0	0	0.56
Tridecene	1309	0.81	0	0
Undecanal	1310	0	0.71	0.66
Hexenyl tiglate-(3E)	1323	0.78	0.36	0
Caryophyllene-(Z)	1325	0	1.92	0
Decyl acetate	1330	0	0.32	0
β-copaene	1335	0	0.77	0
α-guaiene	1339	0	0.65	0
Neryl acetone	1351	0.84	3.56	1.88
α-humulene	1361	0	0.48	0
β-damascenone-(E)	1381	0.67	0.84	0
β-ionone-(E)	1383	1.97	0.99	0.56
Germacrene-D	1386	0	2.16	0
Methyl decyl	1394	0	1.34	0
2-tridecanone	1396	0	0.66	0
Tetradecane	1401	0.45	0	0
Dodecanal	1411	0.82	4.08	0.59
Pentadecane	1501	0.67	0.45	0.43
Tridecanal	1514	4.55	0.72	4.52
Δ-cadinene	1522	0	0.36	0
Nerolidol-(E)	1563	1.26	0.77	0

Table 1: Continued

Table 1: Continued

Species	KI	*gnidium*	*laureola*	
Populations		Megrès	Beni aziz	O. El-Bared
Hexenyl benzoate-(3Z)	1575	4.47	0.96	2.53
Spathulenol	1584	0	0.13	0
Caryophyllene oxide	1589	0	0.36	1.36
Tetradecanal	1615	1.01	1.49	1.09
α-cadinol	1661	0	0	3.29
Heptadecane	1765	1.52	0.46	0
Cyclocolorenone	1751	0	0	1.32
Drimenin	1826	0	0	15.76
Phytol	1842	1.37	0	2.42
Pentadecanone	1844	0	2.64	0
Farnesyl acetone-(5Z, 9E)	1913	0	1.92	0
Methyl hexadecanoate	1926	0	0.21	0
Palmitic acid	1966	20.97	6.19	9.47
Eicosene	2114	12.49	0	0
Phytol acetate-(E)	2115	0	0	4.52
Linoleic acid	2144	6.83	0	2.91
Octadecanol acetate	2146	0	0.88	0
Triacosane	2200	0	0	0.54
Tricosane	2300	1.85	1.74	0
Octacosane	2335	2.11	0.90	1.77
Tetracosane	2399	0.58	0	0
Triacontane	2399	0	0	0.95
Pentacosane	2501	2.96	2.86	2.95
Dotriacontane	2599	0	0	0.48
Tritriacontane	2700	0	0	4.68
Hexacosane	2702	3.67	2.41	0

Table 2
Inhibition zone diameter of *Daphne* species essential oil

Species	*D. laureola*		*D. gnidium*	Gentamicin
Populations	Beni aziz	Oued Elbared	Megrès	
Escherichia coli ATCC 25922	10.8 ± 0.7	8.5 ± 0.5	7 ± 1	14
Micrococcus luteus ATCC 533	6.2 ± 1	8.2 ± 1	0	18
Staphylococcus aureus ATCC 6538	0	0	0	25
Bacillus cereus ATCC 10878	0	0	0	24
Saccharomyces cerevisiae ATCC 763	0	0	0	0

(*) Average of inhibition zone diameter (mm) of three experiments with Standard Deviation

REFERENCES

1. Ullah N, Ahmad S, Anis E, Mohammad P, Rabnawaz H, Malik A. A lignan from Daphne oleoides. Phytochemistry, 1999a; 50: 147–149.
2. Ullah N, Ahmed S, Muhammad P, Ahmed Z, Nawaz HR, Malik A. A dicoumarin glycoside from Daphne oleoides. Phytochemistry, 1999b; 51: 103–105.
3. Riaz M, Malik A. Daphsaifnin, a dimeric coumarin glucoside from Daphne oleoides. Heterocycles, 2001; 55: 769–773.
4. Li SH, Wu LJ, Gao HY, Chen YH, Li Y. A new dicoumarinoid glycoside From Daphne giraldii. J. Asian Nat. Prod. Res, 2005; 7: 839 – 842.
5. Baba K, Yoshikawa M, Taniguchi M, Kozawa M. Biflavonoids from Daphne odora. Phytochemistry, 1995; 38: 1021–1026.
6. Zhang Wei, WeiDong Zhang, TingZhao Li, RunHui Liu, HuiLiang Li, HaiSheng Chen. A new flavan from Daphne odora var. atrocaulis. Fitoterapia, 2004; 75(7-8): 799–800.
7. Liang S, Tang J, Shen YH, Jin HZ, Tian JM, Wu ZJ, Zhang WD, Yan SK. Biflavonoids from Daphne feddei and their inhibitory activities against nitric oxide production. Chem. Pharm. Bull, 2008; 56: 1729–1731.
8. Zhuang L, Seligmann O, Jurcic K, Wagner H. Constituents of Daphne tangutica. Planta Med, 1982; 45: 172–176.
9. Su J, Wu Z, Shen Y, Zhang C, Zhang W. Lignans from Daphne giraldii. Chem. Nat. Compd, 2008; 44: 648–650.
10. Taninaka H, Takaishi Y, Honda G, Imakura Y, Sezik E, Yesilada E. Terpenoids and aromatic compounds from Daphne oleoides ssp. Oleoides. Phytochemistry, 1999; 52: 1525–1529.
11. Zhan ZJ, Fan CQ, Ding J, Yue JM. Novel diterpenoids with potent inhibitory activity against endothelium cell HMEC and cytotoxic activities from a well-known TCM plant Daphne genkwa. Bioorg. Med. Chem, 2005; 13: 645–655.
12. Li Pan, Xiao Feng Zhang, Hai Feng Wu, Li Sheng Ding, A New Daphnane Diterpene from Daphne tangutica. Chinese Chemical Letters, 2006; 17(1): 38–40.
13. Zhang Yilin, Haiqing Zhang, Shineng Hua, Lianghui Ma, Cong Chen, Xiaoyu Liu, Liqun Jiang, Huanming Yang, Peicheng Zhang, Dequan Yu, Yinlong Guo, Xuehai Tan, Jianfeng Liu. Identification of two herbal compounds with potential cholesterol-lowering activity. Biochem. Pharmacol, 2007; 74(6): 940–947.
14. Cottiglia F, Loy G, Garau D, Floris C, Casu M, Pompei R, Bonsignore L. Antimicrobial evaluation of coumarins and flavonoids from the stems of Daphne gnidium L. Phytomedicine, 2001; 8(4): 302–305.
15. Kabouche A, Kabouche Z, Seguin E, Tillequin F, Bruneau C. A phenylethanoid glycoside and flavonoids from Phlomis crinite (Cav.) (Lamiaceae). Biochem. Syst. Ecol, 2005; 33: 813–816.
16. Lopez-Posadas R, Ballester I, Abadıa-Molina AC, Suarez MD, Zarzuelo A, Martíez-Augustin O, Sanchez de Medina F. Effect of flavonoids on rat splenocytes, a structure– activity relationship study. Biochem. Pharmacol, 2008; 76: 495–506.
17. Limem-Ben Amor I, Skandrani I, Boubaker J, Ben Sghaïer M, Neffati MA, Bhouri W, Bouhlel I, Chouchane N, Kilani S, Guedon E, Ghoul M, Ghedira K, Chekir-Ghedira L. Investigation of biological activity of polar extracts isolated from Phlomis crinite Cav ssp. mauritanica Munby Drug. Drug Chem. Toxicol, 2009; 32: 38–46.
18. Harizi Hedi, Fadwa Chaabane, Kamel Ghedira, Leila Chekir-Ghedira. Inhibition of proinflammatory macrophage responses and lymphocyte proliferation in vitro by ethyl acetate leaf extract from Daphne gnidium Tunisia, Cellular Immunology, 2011; 267: 94–101.
19. Mohammedi Zohra. Etude Phytochimique et Activités Biologiques de quelques Plantes Médicinales de la Région Nord et Sud Ouest de l'Algérie. Thèse de Doctorat en Biologie, Université de Tlemecen, 2013; 170p.
20. Yoshitaka Ueyama, Seiji Hashimoto, Hiromichi Nii, Kiyoshi Furukawa. The Chemical Composition of the Essential Oil of Daphne genkwa Sieb. et Zucc. Journal of Essential Oil Research, 1990; 2(5): 247–250.
21. Ilhan Gurbuz, Betul Demirci, Gerhard Franz, Kemal Husnu Can Baser, Erdem Yesilada, Fatih Demirci. Comparison of the volatiles of Daphne pontica L. and D. oleoides Schreber subsp. oleoides isolated by hydro- and microdistillation methods. Turk. J. Biol, 2013; 37: 114–121.
22. He WD, Cik M, Appendino G, Van Puyvelde L, Leysen JE, De Kimpe N. Daphnane-type diterpene orthoesters and their biological activities. Mini Rev Med Chem, 2002; 2: 185–200.
23. He WD, Cik M, Lesage A, Linden IVD, De Kimpe N, Appendino G, Bracke J, Mathenge SG, Mudida FP, Leysen JE, Puyvelde LV. Kirkinine a new daphnane orthoester with

potent neurotrophic activity from Synaptolepis kirkii. J. Nat. Prod, 2000; 63: 1185–1187.
24. Kwon Ki Bang, Cheong-Yong Yun, Chul Lee, Qinghao Jin, Jin Woo Lee, Sang-Hun Jung, Dongho Lee, Mi Kyeong Lee, Jin Tae Hong, Youngsoo Kim, Bang Yeon Hwang. Melanogenesis inhibitory daphnane diterpenoids from the flower buds of Daphne genkwa. Bioorganic & Medicinal Chemistry Letters, 2013; 23(11): 3334–3337.
25. Carney JR, Krenisky JM, Williamson RT, Luo J, Carlson TJ, Hsu VL, Moswa JL. Maprouneacin, a new daphnane diterpenoid with potent antihyperglycemic activity from Maprounea Africana. J. Nat. Prod, 1999; 62: 345–347.
26. Li Pan, Xiao Feng Zhang, Ye Deng, Yan Zhou, Huan Wang, Li Sheng Ding. Chemical constituents investigation of Daphne tangutica. Fitoterapia, 2010; 81: 38–41.
27. Yang YC. Traditional Tibetan medicines, Xining: Qinghai People's Press, 1991; pp 427–429.
28. Farrukh Mansoor, Itrat Anis, Sajjad Ali, Muhammad Iqbal Choudhary, Muhammad Raza Shah. New dimeric and trimeric coumarin glucosides from Daphne retusa Hemsl. Fitoterapia, 2013; 6(88): 19–24.
29. Farrukh Mansoor, Itrat Anis, Ajmal Khan, Bishnu P. Marasini, Muhammad Iqbal Choudhary, Muhammad Raza Shah. Urease inhibitory constituents from Daphne retusa. Journal of Asian Natural Products Research, 2014; 16(2): 210-215.
30. Süntar I, Küpeli Akkol E, Keles H, Yesilada E, Sarker SD, Arroo R, Baykal T. Efficacy of Daphne oleoides subsp. kurdica used for wound healing: identification of active compounds through bioassay guided isolation technique. J. Ethnopharmacol, 2012; 141(3): 1058-1070.
31. Yang YZ, Ranz A, Pan HZ, Zhang ZN, Lin XB, Meshnick SR. Daphnetin: a novel antimalarial agent with in vitro and in vivo activity. Am J Trop Med Hyg, 1992; 1: 15–20.
32. Yesilada E, Taninaka H, Takaishi Y, Honda G, Sezik E, Momota H, Taki T. In vitro inhibitory effects of Daphne Oleoides ssp. Oleoides on inflammatory cytokines and activity guided isolation of active constituents. Cytokine, 2001; 13: 359–364.
33. Yusa K, Oh-hara T, Tsukahara S, Baba K, Taniguchi M, Kozawa M, Tkeuchi S, Hara H, Tsuruo T. Inhibition of human immunodeficiency virus type I (HIV-1) replication by Daphonodrins. Antivir Res, 1994; 25(1): 57–66.
34. Jurd L, Corse Jr J, King AD, Bayne H, Mihara K. Antimicrobial properties of 6,7-dihydroxy-7,8-dihydroxy-,6-hydroxy- and 8-hydroxycoumarins. Phytochemistry, 1971; 10(12): 2971-2974.
35. Xu RS, Gao YS. Recent advances in chemical studies on the active principles from plants for fertility regulation Pure. Appl Chem, 1986; 58: 811-816.
36. Xu H. Pesticidal activities of some important Chinese medicinal plants, Rec Prog in Med Pl. 2010; p. 309.
37. Commission CP. In Chinese Pharmacopoeia, Chinese Medicine Science and Technology Press, China, 2010; 1: 148p.
38. Bellakhdar J, Claisse R, Fleurentin J, Younos C. Repertory of standard herbal drugs in the Moroccan pharmacopoea. J. Ethnopharmacol, 1991; 35: 123–143.
39. Iauk L, Aleo G, Caccamo F, Rapisarda A, Ragusa S, Speciale AM. Antibacterial and antimycotic activities of Daphne gnidium L. extracts. Phytoter. Res, 1996; 10: 166–168.
40. Iauk L, Aleo G, Caccamo F, Rapisarda A, Ragusa S, Speciale AM. Comparative evaluation of antibacterial and antimycotic activities of Daphne gnidium L. leaf and bark extracts. Farmaci & Terapia, 1997; 14: 37–43.
41. Cabrera E, Garcia-Granados A, Macqueda M. Antibacterial activity of coumarin isolated from Daphne gnidium L. Microbios Lett, 1988; 37: 153–159.
42. Burrows G, Tyrl RJ. Daphne L. Toxic Plants of North America, Ed. Ames, Iowa: Iowa State University Press, 2001; pp 1158-1160.
43. Tayoub ghaleb, Amer Abu Alnaser, Motassim Shamma. Microbial inhibitory of Daphne oleifolia Lam. Ethanolic extract. Int. J. Med. Arom. Plants, 2012; 2(1): 161-166.
44. Manojlovic Nedeljko T, Pavle Z Maskovic, Perica J Vasiljevic, Ratomir M Jelic, Marina Z Juskovic, Miroslav Sovrlic, Leka Mandic, Marija Radojkovic. HPLC Analysis, antimicrobial and antioxidant activities of Daphne cneorum L. Hem. Ind, 2012; 66(5): 709–716.
45. Javidnia K, Mojab F, Mojahedi SA. Chemical constituents of the essential oil of Stachys lavandulifolia Vahl. from Iran. J. Essent. Oil Bearing Plants, 2003; 6: 174–178.
46. Darlington CD, Janaki Ammal EK. Chromosome Atlas of Cultivated Plants, George Allen & Unwin, London. 1945.

47. Pastor Díaz J, Fernández I, Díez MJ. Numeros cromosomicos para la flora espanola. Lagascalia, 1988; 15(1): 124-129.
48. Ivanova D, Vladimirov V. Chromosome numbers of some woody species from the Bulgarian flora. Phytol. Balcan, 2007; 13(2): 205–207.
49. Björkqvist I, von Bothmer R, Nilsson Ö, Nordenstam B. Chromosome numbers in Iberian Angiospermes. Bot. Not, 1969; 122: 271-283.
50. Diosdado JC, Santa-Barbara C, Vioque J, Juan R, Pastor J. Números cromosómicos para la flora Española, 691-719, Lagascalia. 1993; 17(1): 173–184.
51. Goldblatt PG (ed.). Index to plant chromosome numbers 1979–1981, Monogr. Syst. Bot. Missouri Bot. Garden, St. Louis, 1984; 8: 1–427.
52. Goldblatt PG, Johnson D.E. (eds.). Index to plant chromosome numbers 1990–1991, Monogr. Syst. Bot. Missouri Bot. Garden, St. Louis, 1994; 51: 1–267.
53. Goldblatt PG, Johnson DE. Index to Plant Chromosome Numbers 1994–1995, Monogr. Syst. Bot. Missouri Bot. Gard, 1998; 69: 1–208.
54. Löve A, Kjellqvist E. Cytotaxonomy of spanish plants. IV. Dicotyledons: Caesalpinacea-Asteracea. Lagascalia, 1974; 4(2): 153–211.
55. Quézel P, Santa S, Nouvelle flore de l'Algérie et des régions désertiques méridionales, 2 Vol., Ed CNRS, Paris, 1963.
56. Lapie G, Maige A, Flore forestière illustrée de l'Algerie, Published by Forgotten Books, Paris, 2013.
57. Masada, Y, Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry, Halsted, Nueva York, 1976, 334p.
58. NIST. Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library, vers. 2.0. fiveash data, USA, 2002.
59. Adams RP, Identification of essential oil components by gas chromatography and quadrupole mass spectrometry, Allured Publ. Corp., Carol Stream IL, 2001.
60. Lograda Takia, Messaoud Ramdani, Pierre Chalard, Gilles Figueredo, Charactreristics of essential oils of Rosmarinus officinalis from eastern Algeria. Global J. Res. Med. Plants & Indigen. Med, 2013; 2(12): 794–807.